Lecture, Stony Brook, April 4, 1984

SIGNIFICANCE OF RESPONSES OF THE GENOME TO CHALLENGE :

I. Early history; genetics. 1921 through 1930.

1. Genetics, Cornell Univ. 1921. Course. Content. Emerson, maize. Greduate course, spring, 1922. Reaction of biologists; histology and embryology=negative.
   Chromosomes: course. relation to heredity. Drosophila by then. Studies with nerve cells (meaning of salivaries not realized until 1934-35.)
   Chromosomes, maize. Even correct number not known.
2. Graduate school, 1923. Focus:relation of chromosomes to genetics with maize.
3. By 1928-29: discovered usefulness of pachytene chrs. at meiosis. Most successful: elongate chromosome bivalents in very large nucleus: room to spread out without too much entanglement.
   Distinguishing chromosomes at pachytene: relative size; position of centromeres (arm lengths); chromomere patterns, linear order (most important)
4. By 1930: could place genes in chr.9 short arm in liniear order. Could determine to fine degree positions of translocation breaks.
5. 1928: Discovery of effect of X-rays on producing mutations: Muller in Drosophila; Stadler with barley. Profound effect on geneticists: could get mutants at will instead of waiting for spontaneous occurrences.

II. University of Missouri, Summers 1931 and 1932.

1. 1938-1931. Stadler; mutations in maize by X-rays.
2. How conducted: Pollen, two sperms, wild-type genes; X-rayed placed on silks of plants having recessive mutants of known types for plants.
3. Kernels from irradiated pollen on these ears. Sown in field, summer 1931. Look through plants for expression of recessive carried by female parent. Assumption: a mutation occurred in irradiated sperm.
4. Request that I examine these plants, summer 1931. Selection of plants to be examined.
5. The results: Many chromosomal rearrangements: inversions, deficiencies, translocations and many complex reordering. Significance, all broken ends fused 2 x 2. No stars.
   Mutants: deficiencies of locus in pollen irradiated.
6. Fall, 1931. Reprint from Berkley; Nicotiana, variegated: due to fragment chromosome lost during some mitoses. Exposed recessive expression in normal chromosomes of complement.
   My response: Could be a ring chromosome: reason, sister strand exchanges between chromatids=after or during chromosome replication. Would make a double-size ring, two centromeres; passage to opposite poles of spindle- left out or something must happen.
   Not without some logic. Variegated plants in field, summer 1931=ring chromosome plants. Had not been examined. Wrote to Stadler asking if he would grow more of the same as that of 1931. I would come to Missouri and examine these plants.
7. All variegated plants had a ring chromose. Behavior of the rings.
   <u>SLIDE 1.</u>

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Significance: Rupture of both chromosome bridges; entrance not close to each other in telophase nucleus; always found one another; fusions 2-by-2 in correct orientation (now-5' .. 3' orientations).

SENSING: what is sensed? how are brokens brought together? what is the over-all mechanism? 2-by2 fusions basic to stability of systems. Used in programming: Example= synapsis of chromosomes at zygotene of meiosis; how started; interlocking of sysnapsed chromosomes; sensing of this; breaks to free ends to untwist; fusions of broken ends, now released; pachytene stage has no interlocked bivalents. Occasional miss at this stage and two interlocked bivalents seen in diakinesis.

Experiment later: entrance of newly broken end from male amd female gametes into zygote: fusion of these broken ends to produce dicentric chromosome. How sensed?

III. What would happen if a single broken end entered a telophase nucleus?

1. Alerted to this in early 1930s in studying a translocation between nucleolus organizer on chromosome 6 of maize and chromosome 5, some distance from its centromere.
2. Studying plants homozygous for this deficiency. Its morphology (Diagram on board.)

Normal bivalent

Homozygous translocation 6-5

3. Terminalization: chiasma to end of arm. The fource behind this. Occurs at diplotene.
4. Normal bivalent: no crossovers centromere to nucleolus. No trouble.
5. Homozygous inversion: many crossovers. Terminalization; to nucleolus edge. Rupture and fusion of ends. Bridge at anaphase; single (2-strad) broken end enters nucleus. What will happen to this end in next replication and mitosis?
6. Available material then: Inversion in chromosome 4. Heterozygous. Crossovers SLIDE 2, diagram. SLIDE 3, Photographs of bridge and fragment; AI, Pro II, AII, TII; spores.
7. Answer to this challenge: No telomere; no quide for replication of free end. The immediate consequence, replication unites chromatids where break occurred in previous anaphase.
   QUESTION: Why does this occur. What is sensed and what mechanism goes into operation?
8. Some answers coming up.

IV. Behavior of broken end in development of gametes and in tissues formed by these gametes after double fertilization.

1. Pollen grains with ruptured end -- non-functional. Long deficiency.
2. To obtain chromosome in gametes with newly ruptured end but nucleus carries genes necessary for functional gametes.
3. The method: SLIDE 4. Diagram chromosome 9 short arm, normal and duplication in reverse orientation. Crossover and consequence SLIDE 5.

4. Development of the male gametes: Tassel; florets, anthers, meiosis in anthers. The breaks, bridges,; spores. SLIDE 6, same as slide 3. Growth of two cell to pollen: generative nucleus division; Pollen grain: onto hairs on silks (wind blown pollen). Pops plug, tube comes out and goes down silks of female plant. Each silk leads to kernel to be carrying the product of meiosis in the female. Dumps sperms into product of one cell of 4 produced by meiotic divisions.

5. Development of embryosac: One cell in structure within kernel-to-be. Meiosis; 4 linear arranged spore. Three degenerate; one enlarges. Basically three mitosis of this haploid spore to produce female gametophyte, or embryo sac. SLIDE 7

6. The sperm and egg fusion; the sperm and endosperm fusions. Genetic constitutions: sperm and egg= zygote; diploid 2n Sperm and two haploid nuclei in haploid embryosac to endosperm, 3n. CENTRAL CELL, its significance. "Double fertilization"

7. Early mitoses in endosperm; later; how ordered. Consequence important as will appear later.

8. Development of embryo and endosperm into mature kernel:

   SLIDE 8; Mature kernel: Photo, SLIDE 9. Independence of embryo and endosperm.

9. Pigment production in kernels. SLIDE 10 COLOR, Ear commercial maize.

   Pigment in aleurone layer: SLIDE 11, photo long section kernel.

10. What happens to single broken end after being delivered to zygote and primary endosperm nucleus: SURPRISE. Broken end from male or female

    a). Endosperm: chromatid type of b.f.b cycle continues throught out endosperm development, as it had in both male and female gametophytes

    b). Broken end "heals" in the embryo. MAKES A NEW TELOMERE. Telomere as stable as any other end. Observed through many plants and plant generations. One used in genetic studies by many labs since 1941. Still being used. I have used many of these carrying altered chromosome constitutions.

    c). Mechanism that senses broken end and insticages mechanism for making a new telomere supported by mutant that will not allow new telomere formation in plant cells. Recessive.

    d). Indicates system for making new telomere not operating in gametophytes and endosperm. Turned on in embryo.

11. Importance of ability to make telomeres at broken ends: many.

    a). Oxytrica, Stylonycia, Tetrahymena, other ciliates. Macronucleus development.

    b). Amplifications in response to poisons: double minutes with methotraxate; virus infections; fragment chromosomes from ruptured chromosomes, etc.

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V. ANOTHER SIGNIFICANT RESPONSE TO THE CHALLENGE OF BROKEN ENDS ENTERING A TELOPHASE NUCLEUS.

1. Completely startling and unexpected result was the activation of previously silent but potentially transposable elements. Their entrance into gene loci and the change in product of gene action and regulation of time and tissue in which this is expressed.

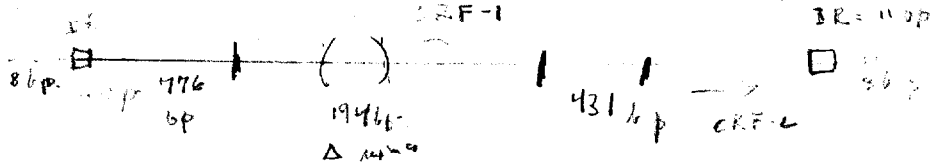
2. Both the chromatid and chromosome type of b.f.b.cycle:involves newly ruptured ends of chromosomes entering telophase nuclei, and the response to these continuing challenges to genome stability.

3. Progeny of cells that began development with the chromosome type of b.f.b cycle: 450 plants; self-pollinated; seedling progeny; the changes in expression of genes, particularly chlorophyll genes. Changes in patterns of gene expression: related to one cell gains what other cell looses. Relates to specific types of altered regulation of gene expression.

4. Isolation and study of different types of transposable elements. Distinctly different elements and families of elements within each system. Two systems best studied at both genetic and molecular level: The Ac-Ds system and the Spm system of elements.

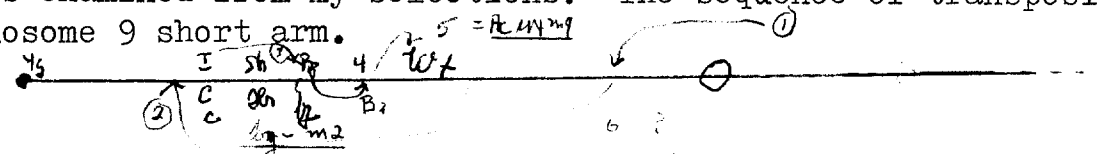
VI. Molecular studies of the Ac system.

1. The organization of the Activator element. (On board) Ac wx-m9



2. The origin of a Ds element. From Ac-wx-m9.

3. The Acs examined from my selections: The sequence of transpositions: Chromosome 9 short arm.



SLIDE 13. Color, I-C; Bz-bz, Sh-sh

4. Ac at wx-m9; wx-m7; bz-m2. Same constitutions, based on restriction fragments and sequencing.

5. Ds at Adh1; at Sh1 ; in genome, not otherwise detected. Ds from Ac-wx-m9. Various types expected. Duplications, insertions into Ds itself; Ds at wx-m1, wx-m6, All different. Arise from changes in Ac, and then can pick up other DNA.

VII. The relation of the inserts to expression of the gene.

1. The Wx gene: Spontaneous mutants. Many are insertions. Relation to position in the gene locus. Oliver Nelson; fine structure analysis by crossing over between each of the isolates; ordering of mutants on basis of crossing over. Ordering of mutants on basis of location of insert, associated with mutants: molecular and genetic evidence the same.

2. The controlling element inserts: Ds-wx-m1, wx-m6, wx-m5, wx-m9 from Ac: Same as Ac at wx-m9. Loss of 194 b.p. only.

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3. Locations and regulations at Wx gene; Sharp expressions of regulation of gene action where the Ac or the Ds element is by the 5" part of the gene. Some examples from Ac wx-m7.

a). Ac, two regulations, relate to inactivity of the transposase gene, a non-clonal, positional information patterns of expression and transposase active: mutational changes giveing clonal patterns of gene expression.

b). Example SLIDE 14. Development of kernel endosperm clearly expressed in clones of deep Wx stain (amylose content) overlying a quite different pattern of lightly amylose staining in individual cells.

c) No transposase action at all. Pattern, non-clonal. SLIDE 15.

d) Same as Slide 15 only transposase activity turned on in single cell, early in development to form sector affecting DNA changes to Wx and to a-m3, A gene under control of transposase activity. SLIDE 16.

e). SLIDE 17, Another pattern of inactive transposase background/ non-clonal pattern. Two halves of same kernel (ground down surface, loss of layer from this).

f). SLIDE 18. Another pattern of inactive m plus few activations.

4. Non-clonal patterns elsewhere: All over in nature. Single alleles of single locus: Ladybird beetle; clover leaf patterns. Combinations of patterns; each allele on its own. Same with maize alleles.

VIII. Expressions of regulation at the Bronze gene; anthocyanin pigment.

1. Bronze gene: expression in plant and in aleurone layer of endosperm.

2. Needed genes for coordination in production of anthocyanin:

A1, A2, C1, C2, Bz1, Bz2, R.

3. R and C2 appear to be regulators. Bz and inactive r or c2 =

Bz enzyme not formed in development of endosperm.

4. bz-m4. Right enzyme made but at wrong time and in wrong gells" Early in starch cells; turns off early.

b). No change with inactive r or c2. Pays no attention to regulators.

c). Ac present; returns to making enzyme at right time in right cells.

d). The significance of this at DNA level.

5. Spm system, Nelsons bz-m13. Products of gene with receptor of Spm transacting products. No Spm present: makes right enzyme but in in wrong cells, initially, then to no enzyme and again, late in kernel development to right enzyme in right cells, probably: aleurone pigmented.
Second product: cross reacting product of Bz gene: about same time and rate as that made by normal Bz gene.

6. Significance of bz-m4 and bz-m13 for regulation of gene action.

IX. Some other evidence of gene regulation given by the controlling elements.

1. Spm. Sp gene product. Turns gene action off (or on, in some cases) No Sp product: gene action turned on (or off in above cases).

2; Programmed change in phase of Spm activity: from no evidence of Spm activity to activity and then to no activity. Regulated.

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3. PRESETTING AND ERASURE. Most important aspect.

a). Difference between plant and animal germlines.

b). Must have special mechanism for going from differentiated cells to gametes.

c). Sequence of events at a gene locus before expression; expression of gene; set back to zero before gamete formation.

d). Not involving a change in DNA at the locus. Something at the locus, however, is so regulated. Methylation on and off is favorite example of gene regulation without DNA change. However, this must be programmed and is in maize. Can be observed when off timed or in harmony with genome.

e). Plants: some basic action to clear genome rapidly: plants from tissue cultures: differentiated to zero modifications.

X. CONCLUSIONS

1. Two double-strand breaks within or entering a nucleus: will find one-one another and broken ends fuse. Makes no difference of mode of breaks: ring chromosomes; entrance of two broken ends into nucleus, from two different sources (gametes and sources), X-ray or other induced breaks; breaks to release interlocked chromosomes at prophase of meiosis; SENSING AND PROGRAMMED RESPONSE.

2. The production of new telomeres at ends of chromosomes in some tissues or plant structures in maize. A programmed response for these parts of plant. SENSING mechanism. Known as "turned-on" in somatic= sporophyte tissues; turned off in gametophyte and endosperm. Useful for understanding "healed ends" in maize; yeast; fragments in ciliate macronuleio Origin of stable "double-minutes.

3. Illustrates mechanisms that activate previously silent, totally silent, elements. These then can transpose to various locations within genome. Their various sequences of transpositions can be followed, from one location to another.

4. Sequences and restriction fragments: show types of organizations within what may be called an initial element. Genetic selections illustrate how these elements change themselves to form a "family" of related elements. With Ac, relatively small number of inactive elements. Many Ds elements with various amounts of DNA and origins of DNA. Have responding sequences at ends. Not known whether these are capable of transposition. I doubt this as it is obvious that elements must be activated in some way when silent in genome.
TEST: Origin of activated Dt elements, their locations, their modes of action.
Totally inactive transposase: could be told if active by dose with Ac; by responses to a allele if Dt present in active state.

5. Entrance of element into gene locus, at some or another location: Regulates time and type of gene expression: Molecular examinations with bz-m4; bzm13 (Ac and Spm regulations). (no Ac + no Spm in bz5)

6. Elements differ strickingly in modes of regulation of gene expression. Ac, Spm, Dt: (a) Dose responses of each for transposase production
(b) Higher dose, later time of activation of m = Ac States of M.

(2) Higher dose, more frequent expressions of m action, no change in time of m action. = Dt
Dose reaction: Dominance of states of this: states late and few evidence of m action; states with late and many responses to m action; states with early responses. Combinations: earliest m actions dominant. = Spm.

-----(b). Components of element that are involved in regulation of action of gene: Ac= two recognized.
Spm= at least 6 recognized. An
**** extraordinarily versitile regulator of gene expression, without change in DNA structure at locus. for both Ac and Spm.

7. Mechanisms of activation: many types of shock, probably. B-f-b cycles chromatid or chromosome certain.
Suspected: virus infections, plants; species crosses in Nicotiana as shown by Carmine-coral variegation. Appearance of same types of variegation in other plants: Antirrhinum: two elements transposable have same terminal sequences as Spm (minor difference) Same sequence at ends in Soybean. Same number of b.p. during insertion in all three cases. SIGNIFICANT.

8. Indicates how polymorphisms originate: activation of elements; their changes in DNA at positions of insertion and also excision, the losses of responive ness to m = new stable modification of locus, new allele of locus and quite new pattern of gene expresion for some. Source of many polymorphisms and of gene expression and of DNA organizations, etc, Insertions that stable, of different sizes, different contents. Rapid production of polymorphisms followed by stabilizations until next shock starts the process over again.

9. Most important revelation: The components responsible for precise types of regulation of gene action associated with inserts: have illustrated their significance in the regulation of gene action over the whole genome. Presetting of the genes and the erasures associated with zygote formation in plants. Some sequence change (Spm component instigated) at gene locus responsible for this.

10. In general, the transposable elements, their origins and their behaviors have told us much about the operation of the genome. Much more should be learned about genome modifications, repetitious DNAs and the sudden changes in their composition and numerical and and positional distributions.

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1. Diagram. Ring chromosome behavior
2. Diagrams, inversion 4 long arm; heterozygous inversion; crossing over.
3. Photos. Single bridge plus fragment. AI, Pro II, AII, TII; Spores.
4. Diagram duplication short arm, chromosome 9/normal chromosome 9. Crossover
5. Diagram. Constitution of crossover: dicentric.
6. Same as Slide 3.
7. Embryo Sac; maize.
8. Diagram. Development of kernel.
9. Photo. Mature kernel, cut longitudinally (Ac wx-m9).
10. Ear, Color. Commercial maize: Indian Corn.
11. Photo. Longitudinal section. Aleurone layer position. Ac-wx-m7.
12. Photo. Two rows of kernels; Dt and activations of Dt.
13. Ear, Color. I-C; Sh-sh, Bz-bz. I Ds Sh Bz/C sh bz + 1 Ac
14. Photo. Long. section kernel. Ac wx-m7. M actions. Dev. of cells shown.

15. Two cut kernels - For clonal Dev. and other (see Fig 4 Dev. Biol Symp 1977)

16. Photo. Long. section kernel. A A . No m action. Non-clonal pattern.
17. Photo. Cross section kernel. Inactive Ac; sector of activated Ac.
18. Photos. Two halves same kernel; Long. section. Early m sector. Inactive elsewhere: pattern of this.
19. Photo. Long. section kernel. (Same as 11). Inactive m plus m action.

Embryology - Sat. days

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